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Spray-dried blood cells as a partial replacement for fish meal in diets for rainbow trout

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GENERAL INTRODUCTION

Because many marine fisheries resources used for preparation of fish meal have reached or exceeded optimum sustained yield and attendant economic response has increased their prices, researchers have given substantial attention to replacements for fish meal in animal feeds. At the same time, there is rising concern over phosphorous (P) content of fish feed because P in excess of the dietary requirements, or in a form that is not digestible, will be eliminated and cause eutrophication problems in aquatic systems. Thus, another reason for finding replacements for fish meal, which is relatively high in total P, is to develop feeds less polluting feeds, that is feeds which contain digestible forms of P and in concentration sufficient to meet requirements for growth.

Concern has been expressed that growth of the aquaculture industry may stagnate unless suitable replacements are found. A shrinking supply but increased demand has sharply increased the price of fish meal. Fish feed costs typically account for over 50% of the costs associated with fish production, and protein ingredients are the major cost item for macroingredients, for example, protein sources typically account for 64% of the feed ingredient cost of Atlantic salmon Salmo salar diets (Prendergast et al. 1994).

Fish-meal based feeds typically contain more phosphorus (P) than fish require. Several commercial trout diets evaluated by Heinin et al. (1993) contained 1.04 to 1.49% P, but rainbow trout Oncorhynchus mykiss require only 0.5% to 0.8% P (Ketola 1991; Ogino and Takeda 1978). Because the majority of P in hatchery effluents comes from fish feed (Lall 1991), feeds should be formulated to contain only available phosphorus at the required level. Heinen et al. (1996) stated that releases of P in effluents range from 58 to 75% of the P present in the feed prior to development of low P feeds for salmonids. Eutrophication problems resulting from fish farm effluents high in P have been noted in freshwaters in both the U.S. and Europe, and in marine habitats affected by net pen culture (Environmental Protection

Agency 1973; Cowey and Cho 1991; Foy and Rosell 1991; Ketola 1991; Ketola et al. 1991; Lall 1991; Ketola and Harland 1993; Ketola and Richmond 1994).

Currently alternatives such as animal by-product meals (blood meals, meat and bone meal, and poultry meals) and plant protein meals are being evaluated by fish nutritionists as a substitute for fish meal proteins (Stickney 1995). Blood meals are potential candidates as replacements for fish meals in fish feeds because they are high in protein (89%, NRC 1993) and lower in P (0.3%) than fish meal (1.7 to 2.9% P). However, iron content of blood meal is high (2,769 mg/kg, NRC 1993). Although heme-bound iron may not be metabolically available to fish, and fish may have regulatory mechanisms to regulate iron balance (Anderson et al. 1996), some concerns have been expressed about diets with high iron content because of potential for lipid oxidation (Hardy 1989) and iron toxicity (Desjardins et al. 1987). However current research and the results of the current study have not substantiated these concerns.

In this thesis, a commercial spray-dried blood cell (SBC) product (AP 301, American Protein Corporation, Ames, Iowa) was evaluated as a partial replacement for herring meal in a diet fed to rainbow trout. SBC are made from blood collected during animal slaughter, which is centrifuged to remove the plasma, and spray-dried to small particle size ("flour"). Growth performance and digestibility of the diets fed to rainbow trout will be compared. Peroxidation of the diets will also be monitored to compare the effect of iron in diets with and without spray-dried blood.

Thesis organization

This thesis is formatted according to the style requirements for publishing in the Journal of the World Aquaculture Society. The first section is a literature review of research on replacements for fish meal, use of blood meals, and the issue of high iron content of fish feeds. The second section presents results of research on use of a commercial spray-dried blood cell concentrate as a manuscript for publication in the Journal of the World Aquaculture Society, of

which I am the primary author and my advisor, Dr. R. C. Summerfelt, is the secondary author. General conclusions of the thesis will follow the second section.

CHAPTER 1: LITERATURE REVIEW

Need for fishmeal replacement

There is a need to replace or reduce fish meal in animal feeds because the marine sources of most fish meal has reached an economical limit (Stickney 1994) and because protein in fish meal is considered more expensive than plant proteins. Fish meal has been the primary source of animal protein for formulating feeds for most species of cultured fish. Fish meal and fish oil constitute about 70% (by weight) of formulated feeds for farmed carnivorous species (Tacon 1996). For example, traditional salmonid diets contained 30 to 70% fish meal by weight (Rumsey 1993). About 12% of the world supply of fish meal is used for aquaculture feed, and 40% is used in poultry feed (Rumsey 1993). However, demand for fish meal for fish diets could grow to 25% of the world supply and actually stagnate further growth of aquaculture production unless substitutes for fish meal are found (Rumsey 1993).

Therefore, development of alternative protein sources is important so the dependency on fish meal can be reduced. Research to reduce and eliminate fish meal in poultry diets has been underway for many years, mostly motivated by the high price of fish meal relative to costs of other protein meals (i.e., soybean, corn gluten, cottonseed, and canola meals). In the midwest, most poultry feeds do not contain fish meal (J. L. Sell, Iowa State University Department of Animal Science, personal communication). The emphasis of contemporary dietary studies on fish has been on two issues: 1) to find substitutes for fish meal, and 2) reduce total P in fish feeds (Ketola and Richmond 1994; Ketola and Harland 1993; Ketola et al. 1991). Cost, anti-nutritional factors, toxicants, or factors which affect palatability or pelleting properties can impose upper limits on feed ingredients in fish diets (Jobling 1993).

In addition to resource depletion, potentially the price of fish used to make fish meal will increase because increasing amounts are diverted into surimi, a minced fish product for human consumption (Stickney 1994). From the view point of trophic efficiency, direct human

consumption of fish is more efficient than feeding fish to fish to produce a human food, because it eliminates the step of aquaculture between wild fish and humans (Stickney 1995).

Because the demand and price of fish meal has increased and the supply of fish meal has decreased, alternative sources of protein for fish diets have been sought. Currently alternatives such as meat and bone meal, poultry meal, and plant proteins are being evaluated by fish nutritionists as a substitute for fish meal (Stickney 1995).

Need for low phosphorus diets

The pollution of receiving waters by fish farm and hatchery effluents, and the eutrophication problems resulting from P discharge have been noted in freshwaters in both the U.S. and Europe, and in marine habitats affected by net pen culture (Environmental Protection Agency 1973; Cowey and Cho 1991; Foy and Rosell 1991; Ketola 1991; Ketola et al. 1991; Lall 1991; Ketola and Harland 1993; Ketola and Richmond 1994). In the U.S., the most widely discussed problem has been with P discharge from Platt River Fish Hatchery, Beulah, Michigan (Ketola et al. 1991; Ketola and Harland 1993; Ketola and Richmond 1994), but similar concerns have been raised elsewhere, especially where salmonids are cultured (Cowey and Cho 1991; Foy and Rosell 1991; Persson 1991).

Rainbow trout Oncorhynchus mykiss require 0.5% to 0.8% available phosphorus (Ketola 1991; Ogino and Takeda 1978). Because fish meal has a higher phosphorus (P) content (2.88%, NRC 1993) than fish require, many commercial diets with a high fish meal content will contain more P than necessary to provide adequate levels of dietary phosphorus. For example, the Oregon Moist Pellet contains about 1.3% phosphorus (Ketola 1991) and the Abernathy diet about 2.2% phosphorus (Ketola 1982). Excess P from fish meal and indigestible P from plant products (phytate P) in the feed is eliminated, mostly in the feces. Soluble orthophosphorus may leach from waste feed and feces, and phosphorus bound to organic molecules with eventually be released through decomposition. The soluble P that enters the water column will enrich downstream waters, causing eutrophication.

In pond culture of channel catfish Ictalurus puncatatus, excess phosphorus in feed can cause water quality problems within the production ponds. Phosphorus stimulates algal blooms that cause problems such as low dissolved oxygen and off flavors in the fish flesh (Robinson et al. 1996). Therefore, low phosphorus diets are also needed in other aquacultural situations in addition to flowing water.

To reduce the P level of hatchery effluents, recent nutritional research has emphasized the development of a low P feed by finding substitutes for fish meal in salmonid diets because the majority of P in hatchery effluents comes from fish feed (Lall 1991). A literature review by Heinen et al. (1996) shows that prior to development of low P feeds for salmonids, releases of P in effluents range from 58 to 75% of the P present in the food.

Estimated blood availability

For obvious health and environmental reasons, livestock blood cannot be discharged in the effluent of abattoir for ruminants and swine (Okuneye and Banwo 1990). Blood collected during slaughter is a valuable resource for recycling into livestock feeds. In the United Kingdom (UK), bloodmeal production has been estimated to be only 22% of the potential yield (Crawshaw 1993).

Information on the amount of bloodmeals produced in the United States is not available. However, it is possible to estimate the potential blood meal production from data on number and size of animals slaughtered and the volume of potential available blood. Collectable blood from livestock has been estimated for several species, ranging from 3% to 6% live weight in bovids and 5% in swine (Warriss 1984). Crawshaw (1993) used a value of 3% live weight to estimate the total amount of blood available in the UK. Using statistics gathered from swine and bovine slaughter at federally inspected abattoirs in 1996 (NASS 1997) and blood loss values for each species (as % live weight), an estimate of almost 1.4 billion kg of blood could be collected from swine and beef in the U.S. (Table 1). In 1996, assuming 20% solids, there was a potential bloodmeal production of 285 million kg.

Table 1. Estimate of total blood available in the United States from cattle and swine slaughtered in federally inspected facilities.

Source	Number Slaughtered[a] (millions of head)	Average live weight (kg)[a]	Total weight slaughtered (millions of kg)	Blood loss, % live weight[b]	Total kg of blood available (millions of kg)
Bovine					
Cattle	35.7	532.1	19,900.7	4.5	895.5
Calves	1.7	154.2	264.2	4.5	11.1
Porcine	90.5	115.2	10,429.6	5.0	521.5
Total					1,428.1

[a]Source: National Agriculture Statistics Service. 1997.
[b]Blood loss values as percent live weight (Warriss 1984).

Blood processing

Blood meal and spray-dried blood are defined by the Association of American Feed Control Officials Inc. (AAFCO 1997) as being "produced from clean fresh animal blood, exclusive of all extraneous materials such as hair, stomach belching, urine, except in such traces as might occur unavoidably in good factory practice." The AAFCO (1997) also defines the general processes for each of the blood products (Table 2).

Historically, blood was dried in a vat, with temperatures reaching 170°C and drying times of more than 24 hours (Crawshaw 1993). Other forms of blood drying are spray drying, flash drying, ring drying, and conventional cooker drying (Hardy 1989). Today, spray-dried blood is the most common form of blood meal (Hardy 1989).

In spray-drying, the blood is initially vacuum evaporated at low temperatures to about 70% moisture, then spray dried. The temperatures reached during spray drying are estimated to be about 190°C for a duration of 10 to 30 seconds (Fang Chi, American Protein Corporation, personal communication). Rotadisc dryers remove moisture from blood in 2 to 3 hours at 100°C and short duration temperatures of 130 to 140°C (Crawshaw 1993). The method of drying the blood is a primary factor affecting the quality of blood meals, but spray-dried and ring-dried blood meals have been identified as acceptable products (Bureau and Cho 1994).

Table 2. Blood processing procedures as defined by AAFCO (1997).

Product Name	IFN	Procedure
Spray dehydrated animal blood	5-00-381	"Moisture is removed from the blood by a low temperature, evaporator under vacuum until it contains approximately 30% solids. It is then dried by spraying into a draft of warm, dry air which reduces the blood to finely divided particles with a maximum moisture of 8% and a minimum crude protein of 85%." The product must be designated according to its minimum water solubility.
Animal blood meal Conventional cooker dehydrated Steam dehydrated Hydrolyzed dehydrated	5-26-005	"The process must be listed as part of the name such as conventional cooker dried, steamed, or hydrolyzed. The product usually has a dark black like color and is rather in soluble in water."
Flash dehydrated animal blood meal	5-26-006	"A large portion of the moisture is usually removed by a mechanical dewatering process or by condensing by cooking to a semi-solid state. The semi-solid blood mass is then transferred to a rapid drying facility where the more tightly bound water is rapidly removed. The minimum biological activity of lysine shall be 80%."
Fresh animal blood	5-25-007	"Blood protein is produced by quick freezing and/or transporting in a chilled state, clean, fresh, whole or dewatered animal blood. If the product bears a name descriptive of its kind, composition, or origin, it must correspond thereto."

Protein digestibility of bloodmeals has been shown to vary between drying methods and drying times for the same method of drying (Meads et al. 1995). Meads et al. (1995) reported apparent ileal digestibilities of crude protein (ADCP) for rats using spray-dried, ring-dried, rotary-dried, and batch-dried blood meals. The regression equation was formulated: ADCP% = 89.8 - 0.266 x drying time (Meads et al. 1995). Spray-dried blood (0.5 minute drying time) ADCP was the highest at 94.6%, while the average for ring dried blood was 87.7%, 48.4% for batch dried bloodmeal (180 to 210 minute drying time), and 84.5% for rotary dried blood

meal (Meads et al. 1995). Only flash-dried blood meal has a minimum lysine bioavailability standard (80%) required by AAFCO (1997).

Nutrient composition of bloodmeals

Traditional sources of proteins in fish feeds are primarily fish meals, but animal byproduct meals and plant protein meals may also be used. Bloodmeals have a high crude protein (CP) content, 87 to 92% (average, 89.4%), compared with herring meal (Table 3) and other fish meals (menhaden, 64.5% CP; white fish, 62.3% CP). Digestible energy of spray-dried blood for rainbow trout is about 4,300 kcal/kg, which is similar to herring meal (4,340 kcal/kg). When replacing herring meal with spray-dried blood on protein basis, 0.805 kg of spray-dried blood replaced one kg of herring meal.

Jobling (1993) stated that animal by-product meals are often rich in lysine and histidine, but deficient in the sulfur-amino acids, methionine and cystine, which is also the case for bloodmeals. The limiting amino acids in fish diets, in order of limitation, are lysine, methionine, and tryptophan (Halver 1994). Lysine is typically limiting in cereal-based animal rations (Crawshaw 1993) and is likely one of the major constraints in formulation of fish diets (Halver 1994). Bloodmeals are rich in lysine (8.4 to 9.0% of crude protein), a desirable quality in formulating fish diets high in plant proteins. The low methionine levels could be increased using other protein sources, or by the addition of commercial crystalline methionine.

Digestibility of blood meals is variable and depends on the processing method used. The crude protein digestibility of spray-dried blood fed to rainbow trout is 99% compared with only 16% for flame-dried blood meal (Cho 1990). Hajen et al. (1993) determined the digestibility of blood meal (from a continuous drying process) when fed to chinook salmon Oncorhynchus tshawytscha to be 29.4% of CP, 31.9% of gross energy, and 34.8% of organic matter, the low digestibility values of blood meal were suspected to be caused by excessive heating of the blood meal during processing.

Table 3. Proximate analysis and amino acid composition of bloodmeals compared with nutrient requirements of rainbow trout and herring meal.

	Nutrient requirement of trout[a]	Herring meal[a]	Whole beef blood[b]	Spray-dried blood[c]	Spray-dried blood[d]	Spray-dried blood[a]
Crude Protein (%)	38	72.0	18.7	87.0	92.0	89.2
ADC Protein (%)		87	97.4		98.8	
Moisture			79.9			
Crude Fat (%)	8.4	8.4	0.3	0.5	2.0	0.74
DE kcal/kg	3,600	4,340			4,636	4,289
Ash (%)			1.1		5.0	
Phosphorus (%)	0.6	1.67		0.2	0.33	0.33
Iron (mg/kg)	60	114		2,000	2,700	2,769
Amino acids[e]						
Arginine	4.0	4.5	4.3	4.4	4.0	4.2
Histidine	1.8	1.7	6.3	6.7	7.5	5.8
Isoleucine	2.4	3.1	1.0	1.2	0.6	1.1
Leucine	3.7	5.2	12.6	12.7	13.4	12.1
Lysine	4.7	5.6	9.7	8.9	9.0	8.4
Met + cys	2.6	2.8	2.5	1.9	1.4	2.6
Phe + tyr	4.7	4.9	10.1	8.5	9.3	9.5
Threonine	2.1	2.9	5.1	3.9	3.6	4.2
Tryptophan	0.5	0.8	-	1.6	1.2	1.2
Valine	3.2	4.3	8.7	9.2	9.2	8.4

[a]NRC (1993).
[b]Asgard and Austreng (1986).
[c]Burgaard (1994).
[d]SBC data provided by American Protein Corporation, Ames, Iowa.
[e]Amino acids as % of protein.

Leucine is quite high in bloodmeals, ranging from 12 to over 13% of protein, while isoleucine is low in relation to requirements. In mammals, an antagonism between the amino acids arises from an excess of leucine in relation to isoleucine and valine (NRC 1993). Excess leucine can act as an anti-metabolite of isoleucine, thereby increasing the requirement for isoleucine (Asgard and Austreng 1986). Considering the dilution effect, high leucine levels in bloodmeals should not be a problem because rainbow trout are highly tolerant to dietary leucine at levels as high as 9.2% of the diet (Choo 1990).

Phosphorus content of spray-dried blood is low (about 0.3%), only 18% of the phosphorus content of herring meal. This is a desirable characteristic when low phosphorus diets are

formulated. Phosphorus in animal by-products is considered to be well digested by poultry (NRC 1994), and P in spray-dried blood is 90% available to swine (ISU 1996). The digestibility of P in blood meal is 81% for Atlantic salmon Salmo salar compared with 52% for P in herring meal (Lall 1991). Plant protein sources are also low in phosphorus and only 60% of the phosphorus is bound in phytate, which is unavailable to fish. Although fish meals have a higher phosphorus bioavailabilty than plant sources, they also have phosphorus content in excess of the dietary requirement of fish.

Bloodmeals (2,700 ppm Fe) contain the highest level of iron of any protein source in the NRC (1993) tables, with exception of crab meal processing residues which is low in protein (32% CP, 4,356 ppm Fe). Iron is a required nutrient, however, high levels of ferrous sulfate in trout diets has been linked to lipid oxidation (Desjardins et al. 1987). Because bloodmeals are typically low in lipids (0.5 to 2.0%) lipid oxidation would not be expected in the meal, but there is a concern about high iron content in high fat fish feeds and reduced disease resistance in fish fed high iron diets is a concern, these issues that are addressed in following sections.

Bloodmeals in monogastric livestock feeds

Compared with research on the feeding and nutrition of poultry and swine, information on fish nutrition is limited. Therefore, insight can sometimes be obtained for the fish nutritionist from information on nutrition of monogastric animals for insight. A review of the blood meal use in poultry and swine feeds is included for this reason.

In the past, bloodmeals have been considered to be unpalatable to livestock and a cause for reduced performance, therefore, the incorporation rate was limited to 3 to 6% of the diet (Crawshaw 1993). The low availability of lysine in overheated blood meals is likely to blame for poor growth. Reevaluation of bloodmeals has shown that amino acid imbalances were responsible for the depressed growth. Pig diets containing blood meals that were supplemented with isoleucine had improved performance (King and Campbell 1978). Marginal isoleucine levels can become deficient when leucine is in excess (NRC 1994).

For many years, fish meal was considered an essential component of poultry feeds to provide "unidentified growth factors", and was typically included in poultry rations at 2 to 10% (Firman 1994). Due to fishy flavor imparted in the meat and eggs of poultry fed higher levels of fish meal, inclusion levels tended to be at the lower end of that range (Firman 1994). Now, fish meal addition to poultry diets is considered unnecessary because of the discovery that vitamin B_{12} and selenium were provided by fish meal, and that amino acids must be balanced properly (Firman 1994).

Spray-dried porcine plasma (SDPP) and spray-dried blood (SDB) have been shown to improve the performance of early weaned pigs (de Rodas et al. 1995; Kats et al. 1994). SDPP which replaced dried skim milk in diets fed to early weaned pigs improved growth rates during day 0 to 14 postweaning by increasing feed intake with little or no change in feed to gain ratios (de Rodas et al. 1995; Kats et al. 1994). SDPP at levels of up to 10% were found to improve performance (Kats et al. 1995). Methionine supplementation of diets fed to early weaned pigs was necessary to achieve maximum growth when SDPP was used (Kats et al. 1994).

Kats et al. (1994) stated that SDB is a beneficial protein source because incorporation in feed stimulates feed intake by early weaned pigs. Spray-dried blood meal is less expensive than SDPP, therefore a blend of the two products (7.5% SDPP : 1.6% SDB) is more economical and palatability is increased (Kats et al. 1994). Another feeding regime involves feeding a diet containing spray-dried porcine plasma for the first week of feeding, then in the second week replace the plasma with spray-dried blood cells (Dean R. Zimmerman, ISU Department of Animal Science, personal communication).

The mechanism by which the blood products increase feed intake of early weaned pigs has not been determined (de Rodas et al. 1995; Kats et al. 1994). de Rodas et al. (1995) concluded that IGF-I was not readily absorbed or that the amount of SDPP fed was not high enough to increase circulating IGF-I concentrations.

Recommendations by ISU (1996) for the use of SDB meal in swine rations for 20 kg and larger growing pigs or gestating or lactating females is 0 to 5% of the diet. Based on the energy, available lysine content and phosphorus content, bloodmeal out ranks fishmeal and soybean meal as a protein source in pig feeds (Crawshaw 1993; ISU 1996).

Liu et al. (1989) stated that nutritionists are reluctant to incorporate blood meal in turkey diets because of the amino acid balance, and concerns over the availability of blood meals. True amino acid availability's of ring-dried and spray-dried blood fed to turkeys have been determined by Liu et al. (1989). Total available amino acids (TAAA) was higher in spray-dried blood for all amino acids than ring-dried blood.

In some European countries, dietary nitrogen levels have been reduced by using low protein diets and relying heavily on crystalline amino acids to meet dietary requirements (Crawshaw 1993). However, studies have shown that maximum growth and feed efficiency is not achieved using these low protein rations. Highly available protein sources, such as spray-dried blood, would be desirable for use in situations where environmental degradation is a concern.

Bloodmeals in fish feeds

Animal by-products such as glands from slaughtered livestock were heavily used when fish culturists switched from natural food organisms (Piper et al. 1982). Water pollution was a problem in using these crude feeds. Mixtures of spleen, liver, and salt extruded in a meat grinder made a more cohesive feed which reduced water fowling (Piper et al. 1982). These early organ based fish feeds would naturally contain blood present in the organs, so the use of blood is not necessarily a new concept to fish feed formulation.

Salmonids

Fowler and Banks (1976) reported that spray-dried blood meal at 5.8% of the diet, was satisfactory as a partial replacement for fish meal in the Abernathy diet fed to chinook salmon Oncorhynchus tshawytsha. However, liver abnormalities occurred when SDB was fed at 17.5% of the diet, though weights of the fish were not affected. Fowler and Banks (1976)

also noted that at 5.8% of the diet, SDB acted as an excellent pellet binder. Those diets were made in a compaction-type pellet mill without steam conditioning.

Asgard and Austreng (1986) found that whole beef blood could replace 50% of the raw fish or fish offal (about 20% of the protein) in moist salmonid feeds (44 - 48% moisture) without reducing performance or health of the fish. The whole blood, preserved by addition of formic acid, had a calculated apparent protein digestibility of 97%. Organoleptic evaluation of the trout and salmon showed no difference between the fish fed either diet.

Rasmussen (1994) found that 1 kg of spray-dried blood could replace 1.2 to 1.3 kg of LT herring meal in Atlantic salmon diets with levels of spray-dried blood not exceeding 5.0% of the diet. In that study, test diets contained 0, 2.4, 4.8, and 9.5% spray-dried blood, but at the 9.5% level growth was reduced. Dietary iron was 114 ppm in the diet without SDB, 168 ppm in the 2.4% SDB diet, 210 ppm in the 4.8% SDB and 307 ppm in the 9.5% SDB diet. The study design could not distinguish direct effects of total iron content from fat oxidation. Peroxide values, which are indicators of fat oxidation (rancidity) were not reported on individual diets, but ranged from 3.6 to 8.9 meq/kg extracted fat, which Rasmussen (1994) stated indicated a slowly proceeding oxidation.

Luzier et al. (1995) evaluated spray-dried blood as a partial replacement for herring meal in a diet for rainbow trout at 5.7, 11.4, and 22.7% of the diet. Feed conversion and growth of rainbow trout fed the SDB diets was as good as the herring meal-based diet at the end of the 12-week study. The P content of the SDB diets ranged from 0.84 to 1.22% compared with 1.36% for the herring meal diet, thus the SDB diets reduced the amount of phosphorus in the water by 33 to 47%. Phosphorus digestion was greater in the diet with 22.7% SDB than the other diets.

Other species

Gallagher and LaDouceur (1995) found that spray-dried blood could replace 10 to 25% of the fish meal protein in palmetto bass Morone saxatilis × M. chrysops diets with no reduction

in survival or performance. However, at a 50% replacement of fishmeal protein, survival, feed efficiency, and weight gains were significantly depressed. Sullivan and Reigh (1995) estimated the digestibility of blood meal (IFN 5-00-381) to be 78% for dry matter and 86% for crude protein, which was not statistically different from the digestibility of menhaden fish meal.

Disease transmission through feed

The use of animal blood products in feeds may raise concerns that diseases could be transmitted from infected animals used to obtain the blood products to other livestock, fish, or to handlers of the feed or feed ingredients (i.e. feed manufacturer or the end user of a feed containing blood products). Heat used in ring drying and spray drying blood meals may inactivate the pathogens. Most kinds of swine disease should be heat inactivated and there are few zoonotic diseases of ruminants and swine (John Vahley, ISU College of Veterinary Medicine, personal communication). Salmonella could be a transmitted through the blood, however, a septicemic animal would not be allowed into a slaughter plant (John Vahley, ISU College of Veterinary Medicine, personal communication).

Because of the bovine spongiform encephelopathy (BSE) problem in England, the European Community has banned specified risk materials (SRMs) from cattle and sheep from entering feedstuff manufacturing processes because of the potential risk of transmission of BSE (House 1997). SRMs are cattle and sheep parts including skulls, eyes, brains, tonsils, and spinal columns (House 1997). The U. S. Food and Drug Administration has banned the use of any mammalian protein, excluding pure pork and horse which are not known to get BSE naturally, in ruminant feeds (Associated Press 1997). However, mammalian parts used in pet, chicken and hog feed are not included in the ban.

Dietary iron requirements for fish

Function of iron

Iron is a component of heme, hemo enzymes, and non-heme compounds which are essential to life sustaining reactions. Lall (1989) and Lovell (1989) reviewed the function and metabolism of iron. Iron binds oxygen for transport in the heme proteins hemoglobin and myoglobin. Hemo enzymes contain iron, which in the cytochrome enzyme system, regenerates ATP, and in catalase and peroxidase remove oxygen from hydrogen peroxide. The non-heme compounds transport (transferrin) and store iron (ferritin) in the body.

Iron content and hemoglobin content of fish is lower than mammals (van Dijk et al. 1975). The whole body iron content of rainbow trout is 0.35 μmol Fe/g wet weight compared with 1.0 in humans and the proportion of hemoglobin iron as a percent of total iron is 49% in trout versus 65% in humans (van Dijk et al. 1975). In humans, red blood cells contain 33% hemoglobin, and hemoglobin is 0.347% iron (Crosby 1978).

In humans, excess iron must be placed in storage. Iron is transported by a carrier molecule, transferrin, and stored in tissues by the protein ferritin which aggregates to form hemosiderin (Crosby 1978). Transferrin has been identified in several fish species (Lall 1989). Maintenance of iron balance in the human is in the intestines ability to stop absorbing dietary iron when body iron is adequate (Crosby 1978). In the presence of excess iron, ferritin synthesis is stimulated.

Absorption of iron

Few authors have reported the mechanism used by fish to regulate body iron stores. Most iron is absorbed at the intestinal mucosa, but the gill membrane may also absorb iron, while a small amount of endogenous iron is lost in the urine and feces (Lall 1989). Andersen et al. (1996) suggested that Atlantic salmon adapt to the dietary iron content of the feed to regulate body iron.

Salmon fed a purified diet with only 11 ppm Fe had 41% lower whole body iron concentration after four weeks of feeding , and an additional 12 % less after 12 weeks (Andersen et al. 1996). Andersen et al. (1996) stated that this trend was a result of the fishes previous adaptation to a high dietary iron intake, then as the fish readjusted to the lower iron diet, iron absorption increased to account for the reduced iron stores. However, hepatic iron stores decreased throughout 16 weeks on a low iron diet. Hepatic and whole body iron content remained constant in salmon fed 100 to 400 ppm Fe diets. Rasmussen (1994) found that liver iron was the same for Atlantic salmon fed practical diets containing 210 and 307 ppm Fe, while diets containing 114 and 168 ppm Fe had lower hepatic iron concentration. Andersen et al. (1996) proposed that these studies show evidence that fish are able regulate iron metabolism. However, Desjardins et al. (1987) found that rainbow trout fed diets ranging in iron content from 25 to 5,934 mg Fe/kg had body iron contents ranging from 24 to 153 mg Fe/kg dry weight.

Ascorbic acid (vitamin C) and iron have antagonistic relationships in fish diets. Increased dietary iron has been found to decrease hepatic ascorbic acid content of Atlantic salmon (Andersen et al. 1996). Ascorbic acid deficiency in rainbow trout has been observed to reduce serum iron, hematocrit, and hemoglobin levels (Hilton et al. 1978). Iron absorption efficiency is increased by ascorbic acid, which is also a iron chelator and chemically reduces ferric iron (Morris 1977).

Andersen et al. (1996) also found that dietary iron is antagonistic to whole-body manganese content of Atlantic salmon. Manganese competes for absorption sites with iron, a high intake of manganese can interfere with iron absorption (Morris 1977). Dietary excesses of either mineral could reduce the absorption of the other.

Iron requirement

Iron requirements have been determined for several fishes (Table 4). The iron requirement increases during growth periods when blood volume and hemoglobin mass are increasing

(Crosby 1978), thereby changing throughout the life stages of the fish. Tacon (1990) suggested that dietary iron requirement decreases from 60 mg/kg in the fry stage to 30 mg/kg for grower stages of both carnivorous and omnivorous fish reared in intensive aquaculture systems and 60 mg/kg for brood fish.

Table 4. Summary of minimum dietary iron requirements of several fish species.

Species	Iron requirement (mg/kg diet)	Source
Rainbow trout	60	Desjardins (1985)
Atlantic salmon	60-100	Andersen et al. (1996)
Channel catfish	30	Gatlin and Wilson (1986)
Common carp	150	Sakamoto and Yone (1978)
Eel	170	Nose and Arai (1978)

Dietary iron deficiency causes microcytic hypochromic anemia in fish (Steffens 1989). The anemic condition has been characterized by reduced hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin (Lim et al. 1996). To lower the hemoglobin iron content of Atlantic salmon for a bioavailibility assay, a low iron purified diet containing 13.1 ppm iron was fed for 20 weeks (Lall et al. 1996). In a 24 week study, Desjardins (1985) found that feeding a semi-purified diet containing 39 ppm iron lowered the hematocrit values for rainbow trout compared with trout fed diets containing 74 to 241 ppm iron. Reduced feed consumption was observed in channel catfish after only two weeks of feeding a non-iron supplemented diet (9.2 ppm iron) (Lim et al. 1996).

Iron is present in many feedstuffs and water can also provide some iron, though levels in solution (ferrous iron is water soluble, but it is quickly converted to ferric iron in the presence of oxygen and then precipitated) are typically low (Lovell 1989). Typical salmonid feeds contain 200 to 600 ppm iron (Lall 1991). Iron levels in feedstuff are variable because of contamination from ferrous metal during processing (Lall 1989). The bioavailibility of iron is reduced by high dietary intake of phosphate, calcium, phytates, copper, and zinc (Tacon 1990).

Iron contained in plant feedstuffs is of low availability compared with animal byproduct sources, therefore an iron supplement is recommended in plant based fish feeds (Lovell 1989). Bioavailability assay, comparing the availability of iron in ferrous sulfate and spray-dried blood meal, measured by hemoglobin regeneration, indicated no difference between the two forms of iron (Lall et al. 1996). Inorganic iron and iron-protein compounds must be reduced to the ferrous state and released from the conjugate before absorption (Lall 1989). Lim et al. (1996) found that addition of 20 mg Fe/kg from iron methionine or ferrous sulfate in a basal diet containing 9.2 ppm iron was effective in preventing iron-deficiency anemia in channel catfish.

Blood characteristics and tissue concentrations are used to estimate dietary iron requirements in salmonids because weight gains are not affected (Andersen et al. 1996), however, growth was reduced in channel catfish fed iron deficient diets (Gatlin and Wilson 1986). Atlantic salmon fed 11 to 409 mg/kg dietary iron did not differ in weight gains, but liver iron and hematological parameters varied with iron supplementation (Andersen et al. 1996). Rasmussen (1994) reported that stored iron in Atlantic salmon was similar despite wide differences in iron intake (210 and 307 mg/kg). In salmonid studies, iron deficiency does not appear to reduce growth, but channel catfish growth is negatively affected (Gatlin and Wilson 1986; Lim et al. 1996)

In one study, iron toxicity of rainbow trout was reported to be in the range of 86 to 265 mg Fe/kg diet, however these diets also contained high levels of malonaldehyde indicating the lipids were heavily oxidized and the toxicity problem may have been caused by rancid fats (Desjardins et al. 1987). Further studies by Desjardins et al. (1987) found toxicity symptoms developed at 1,380 mg/kg dietary iron when fat rancidity was low (less than 10μg malonaldehyde/g diet).

Potential problems of high iron diets

Lipid autoxidation

Autoxidation, also known as atmospheric oxidation, is the reaction of greatest importance to fish feed manufacturers (Hardy 1989). Carotenoid pigments, tocopherols (vitamin E), and polyunsaturated fatty acids (PUFA) are subject to oxidation (Hardy 1989). Because PUFAs are a dietary component and function in fluidity of biomembranes and are most susceptible to autoxidation, autoxidation of these ingredients is detrimental to fish health. Fish diets typically contain high levels of polyunsaturated fatty acids (PUFA), which are highly susceptible to oxidation reactions when exposed to air (Sargent et al. 1989). The presence of oxidized oils will cause the feed to be off-flavor, reducing consumption (i.e. palatability problem) and create undesirable flavors in the fish (Piper et al. 1982). To prevent autooxidation, it is generally recommended to not store feed longer than 90 to 100 days, and for less time when feed is stored in sub-optimal conditions (Piper et al. 1982).

The process of autocatalytic autoxidation involves three steps, the initiation step is the formation of a free radical (Figure 1). Free radicals formed during the initiation step react with oxygen to form more free radicals, hence the term autocatalytic. The reaction is terminated when stable end products are formed. Catalysts in the initiation step include: light, UV radiation, divalent cations (iron and copper) (Hardy 1989), chlorophyll and hemoglobin (Rehulka 1983 in Steffens 1989). Iron is a catalyst of lipid oxidation, ferrous iron being more active than ferric iron (Jobling 1993). Lipids with an initially high peroxide value have been shown to have a predisposition to oxidation by high iron (ferrous) diets (Desjardins et al. 1987). However, heme bound iron maybe less liable than iron sulfate to initiate the auto-catalytic process, perhaps by binding oxygen available to react in autooxidation.

A strong relationship between peroxide values and poor growth of young Atlantic and coho salmon was demonstrated by Ketola et al. (1989). Desjardins et al. (1987) stated that quality of fish oil may be an important factor influencing the lipid oxidation arising from ferrous

Initiation:

$$RH + O_2 \xrightarrow{\text{Catalyst}} R\bullet + \bullet OOH$$

$$RH + \text{Initiator} \longrightarrow R\bullet + H\bullet$$

Propagation:

$$R\bullet + O_2 \longrightarrow RO_2\bullet$$

$$RO_2\bullet + RH \longrightarrow RO_2H + R\bullet$$

Termination:

$$R\bullet + R\bullet \longrightarrow R\text{-}R$$

$$RO_2 + R\bullet \longrightarrow RO_2R$$

$R\bullet$ = Free radical

RO_2 = Peroxy radical

Figure 1. Free radical formation in oxidizing lipids (Hardy 1989).

sulfate addition to the diet. Desjardens et al. (1987) found that rainbow trout fed diets containing 25 to 86 mg Fe/kg had significantly fewer mortalities than trout reared on diets containing 265, 1334 or 5934 mg Fe/kg. However, in that experiment, the oil in the diets had been oxidized and malonaldehyde concentrations ranged from 68 to 160 µg/g (Desjardins 1987). The trout fed the diets containing iron in excess of 86 mg/kg consumed less feed (Desjardins et al. 1987).

Peroxides can damage cell membranes in fish (Jackson 1988), therefore, feeding oxidized or rancid feeds should be avoided. Defense mechanisms exist within the body to prevent the

peroxidation of body lipids. Glutathione peroxidase and catalase are two enzymes which prevent the initiation of the peroxidation chain reaction (Jobling 1993). The function of glutathione peroxidase is dependent on adequate supplies of selenium in the diet. The tocopherols (vitamin E) and ascorbic acid are chain-breaking antioxidants which are synergistic in that ascorbic acid regenerates vitamin E (Jobling 1993). Vitamins E and C and selenium requirements of the fish are dependent on the susceptibility of the polyunsaturated fatty acids to the peroxidation reactions (Jobling 1993). Therefore, in diets which contain high levels of iron, particularly ferrous sulfate, it is important to provide adequate vitamins E and C and selenium. The ability of erythrocytes to withstand peroxide deterioration of membranes is reduced by vitamin E deficiency (Lovell 1989).

Chain-breaking antioxidants are compounds which intercept peroxyl radicals before they react with other PUFAs (Kaur and Perkins 1991). Vitamin E is one such compound that occurs naturally. Ascorbic acid also reduces oxidation by reacting with oxygen to deplete oxygen available to react in autoxidation (Kaur and Perkins 1991).

The addition of antioxidants can protect the nutritive value of lipids and vitamins (vitamin E) and other oxidation sensitive nutrients from oxidative damage and prevent the formation of toxic peroxides (Jackson 1988, Lovell 1989). Antioxidants are typically added to fish oils during manufacture to prevent oxidation (Hardy 1989). Synthetic antioxidants, such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), are phenolics that prevent oxidation by reacting with free radicals (Hardy 1989). Metallic pro-oxidants can be chelated by ascorbic acid, phytic acid, tartaric acid, oxalic acid, and ethylenediaminetetraacetic acid (EDTA) (Hardy 1989). Naturally occurring antioxidants in many lipid sources inhibit autoxidation during a period called induction time (Hardy 1989), at the end of the induction time autoxidation increases unchecked (Hardy 1989). The inclusion level and the type of antioxidant can affect the stability of the dietary lipids during processing (Desjardins et al. 1987).

Disease resistance

Iron is an essential nutrient for the growth of microorganisms as well as animals and is necessary in maintaining the epithelial barriers of the host organism (Lall 1991). Excess iron suppresses chemotaxis, phagocytosis, and microbicidal action of mononuclear and polymorphonuclear leukocytes (Weinberg 1993). Weinberg (1993) stated there is abundant research on humans linking excess iron to the development of cardiomyopathy, athropathy, various endocrine and neurological diseases, and neoplasia.

Nutritional immunity refers to a host's ability to withhold iron from microbes (Weinberg 1974); more recently, this is termed the iron withholding defense system. The host has several ways of withholding iron from invading microbes (Weinberg 1993), if iron is limited, the host defense is strengthened (Weinberg 1974). Weinberg (1993) stated the host has four defense mechanisms to withhold iron: 1) mobilizing iron binding proteins at invasion points, 2) reducing iron levels in body fluids and in diseased sites, 3) removing non-heme iron from invading cells, and 4) synthesizing immunoglobulins to microbial surface proteins involved in iron acquisition.

Plasma iron levels are considered to be sufficient for microbial growth. Two components which limit the availability of iron are transferrin, which is extracellular, and ferritin, which is intracellular (Weinberg 1974). Aerobic and facultative microbes synthesize siderophores which are phenolates or hydroxymates that solubilize and assimilate metal (Lankford 1973). The siderophores compete with transferrin and ferritin for iron. Siderophores have a high binding affinity to iron and are capable of supplying this iron to bacteria (Wolf and Crosa 1986). Siderophore production has been shown to exist in the fish pathogen Vibrio anguillarum when Atlantic salmon were infected (Wolf and Crosa 1986). In their study, a mutant strain could not take up the siderophore and did not grow due to a lack of iron. However, the siderophore was available to other bacteria. In preliminary experiments, Lall

(1991) found that V. anguillarum was suppressed at dietary iron concentrations of less than 30 mg/kg.

Weinberg (1974) predicted that the number of microorganisms required to produce disease or death is reduced when excess iron is present within the animal and that microbial multiplication will be greater. Weinberg (1974) cited evidence from studies that the host may become hypoferrmic upon microbial invasion. The hypoferrmic state has been shown to occur by two mechanisms: 1) reduced intestinal absorption of iron and 2) larger stores of iron in the livers (Weinberg 1974). It has not been established whether these mechanisms are used by fish.

Ravndal et al. (1994) found high serum iron concentrations in farmed Atlantic salmon were correlated with mortality of vibriosis-infected. They also reported that sexually mature Atlantic salmon had higher serum iron concentrations and lower survival rates when exposed to vibriosis than immature fish.

Goksoyr et al. (1994) demonstrated that a high iron diet (187 ppm Fe) fed to Atlantic salmon did not result in a general depression of cytochrome P450 sub families, P450 1A1 decreased while P450con remained stable, monooxygenase was also reduced. It was speculated by Goksoyr et al. (1994) that these observations in the P450 system may be related to the reduced disease resistance of Atlantic salmon fed high doses of iron (Rorvik et al. 1991 in Goksoyr et al. 1994).

Sealey et al. (1997) studied the relationship between the level and source of iron, and the immune response of channel catfish against Edwardsiella ictaluri. Channel catfish fed iron deficient diets were more susceptible to disease than fish fed diets containing 30 mg Fe/kg, however, iron supplementation of 180 mg ferrous sulfate/kg (possible iron overload for channel catfish) may have increased susceptibility to E. ictaluri.

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CHAPTER 2: SPRAY-DRIED BLOOD CELLS AS A PARTIAL REPLACEMENT FOR FISH MEAL IN DIETS FOR RAINBOW TROUT ONCORHYNCHUS MYKISS

A paper to be submitted for publication in the Journal of the World Aquaculture Society

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Abstract

Traditionally, blood meals used in fish feeds have been flame-dried or spray-dried blood products, however, because of strong commercial markets for spray-dried plasma (e.g., feeding juvenile pigs), the plasma and cellular fractions are routinely separated and processed as distinct products. Thus, spray-dried animal blood cells (SBC) are an available and less expensive product than spray-dried blood meal. The SBC (AP 301™) product used in the present study is manufactured by removing the plasma from the animal blood and then spray-drying the blood cell concentrate. This product is a high protein (93.7%), high lysine (9.0% of protein), and highly digestible (crude protein digestibility coefficient, 99%) feedstuff that is also low in phosphorus (0.33%). These nutritional qualities make it a desirable candidate for partial replacement of fish meal content in fish feeds. The objective of this study was to evaluate growth of juvenile rainbow trout Oncorhynchus mykiss (81.4 mm initial length and 6.4 g initial weight) fed a reference diet containing 47.5% herring meal (67.3% protein) and a test diet containing 34.5% herring meal and 8.75% SBC, both formulated to be isonitrogenous (48.5 and 48.0% protein) and isocaloric (3,977 and 3,927 kcal/kg digestible energy). At the end of the 12-week feeding trial, differences in performance were not statistically significant ($P > 0.05$) between the two treatments. Trout fed the reference diet gained 1.0 mm/day and 0.69 g/day compared with 0.96 mm/day and 0.65 g/day for the fish fed the test diet. Feed to gain

ratios were 0.98 and 1.02 for the reference diet and test diet, respectively. Protein and fat composition of the body increased from beginning to end of the study whereas moisture decreased. Apparent protein digestibility was 89.7% for the diet containing SBC compared with 88.1% for the reference diet, a trivial but statistically significant difference ($P = 0.02$). The higher levels of iron in the diet containing SBC did not have any adverse affect on trout growth or feed conversion. Initial peroxide values of the diets were low, 1.1 and 1.8 mEq/kg extracted fat in diet 1 and 2, respectively, and decreased during the study to 0.52 and 0.82 mEq/kg extracted fat in diet 1 and 2 after 12 weeks. Iron content of the test diet containing SBC was 433 mg Fe/kg, compared with 291 mg Fe/kg in the reference diet. In spite of the high iron content of the diet with SBC, whole body iron content of the fish decreased in both treatment groups in the 12-week experiment from 23.3 mg Fe/kg initially, to 16.5 mg/kg in the reference group, and to 18.4 mg/kg in the test group at the end of 12 weeks. The diet formulated with 8.75% SBC replaced 27.4% of the fish meal and reduced the phosphorus content of the diet by 29.3%. The study suggests that further research is needed on regulation of iron absorption and metabolism of iron-containing compounds, especially related to availability of heme iron in blood meals compared with ferrous sulfate. There is also a need to evaluate effects of dietary iron on the immune response.

Introduction

Many commercial salmonid diets have a high fish meal content, typically containing 30-70% by weight (Rumsey 1993), but relative to plant proteins, fish meal is an expensive ingredient, and the harvest of many marine species used to make fish meal has reached an economical limit (Stickney 1994). Although only 12% of the world supply of fish meal is used for aquaculture feed, demand could grow to 25% of the world supply and actually stagnate further growth of aquacultural production unless substitutes for fish meal are found (Rumsey 1993).

Recent nutritional research has emphasized the development of diets with reduced phosphorus (P) content and the evaluation of substitutes for fish meal in salmonid diets (Ketola

and Harland 1993; Ketola and Richmond 1994; Luzier et al. 1995). Rainbow trout require between 0.5% to 0.8% available P in their diet (Ketola 1991; Ogino and Takeda 1978), but, typically, diets with a high fish meal content have had more total phosphorus than needed. Available P content in excess of nutritional requirements and all indigestible phosphorus in the feed are eliminated in the feces, which results in high phosphorus content in fish hatchery effluents. Because the majority of phosphorus in hatchery effluents comes from fish feed (Lall 1991), to reduce the pollution potential of hatchery effluents the P content should be reduced to only the minimum available required by the fish. Reducing the amount of herring meal, which contains 1.67% P (Table 1), in fish feeds with low phosphorus, high protein ingredients would reduce the phosphorus content of the diet.

Traditionally, blood meals used in fish feeds have been flame-dried or spray-dried blood products, however, because of strong commercial markets for spray-dried plasma (e.g., feeding juvenile pigs), the plasma and cellular fractions are routinely separated and processed as distinct products. Thus, spray-dried animal blood cells (SBC) are an available and less expensive product than spray-dried blood meal. The SBC (AP 301™) product used in the present study is manufactured by removing the plasma from the animal blood and then spray-drying the blood cell concentrate. Spray-dried blood is a high protein, (92%) high lysine (9.0% of protein content) feedstuff, and contains only 0.33% P. Also, SBC equals or exceeds the amino acid requirements of rainbow trout for all essential amino acids except isoleucine and methionine-cystine. These nutritional qualities make it a desirable candidate to reduce fish meal content of the fish feeds.

Although blood by-products have many good qualities, they have a high iron content. The iron content of spray-dried blood cells (SBC, 2,700 mg/kg) is 23.7 times greater than herring meal (114 mg/kg) and 19.3 times greater than soybean meal (140 mg/kg)(Table 1). Increasing iron concentration of rainbow trout diets may increase lipid oxidation (Desjardins et al. 1987). Trout diets typically contain fish oil as a source of energy and essential fatty acids.

Table 1. Proximate analysis and amino acid composition of herring meal, SBC (APC 301), and soybean meal compared with nutrient requirements of rainbow trout.

	Nutrient requirements of trout[a]	Herring meal[a]	Soybean meal[a]	Spray-dried blood cells[b]
International Feed Number	—	5-02-000	5-04-612	5-00-381
Crude Protein (%)	38	72.0	50	92.0
Apparent Protein Digestibility (%)	89.5	87	83	98.8
Crude Fat (% diet)	—	8.4	1.0	2.0
Digestible energy kcal/kg	3,600	4,340	2,934	4,483
Phosphorus content (%)	0.6	1.67	0.64	0.33
Iron (mg/kg)	60	114	140	2,700
Amino acids[c]				
Arginine	4.0	4.5	7.3	4.0
Histidine	1.8	1.7	2.4	7.5
Isoleucine	2.4	3.1	2.3	0.6
Leucine	3.7	5.2	7.3	13.4
Lysine	4.7	5.6	3.2	9.0
Methionine + cystine	2.6	2.8	2.9	1.4
Phenylalanine + tyrosine	4.7	4.9	8.4	9.3
Threonine	2.1	2.9	3.8	3.6
Tryptophan	0.5	0.8	1.4	1.2
Valine	3.2	4.3	5.1	9.2

[a]Values from NRC, 1993.
[b]Spray-dried blood cells (APC 301); data provided by American Protein Corporation, Ames, Iowa.
[c]Amino acids as % of protein.

Autoxidation of fish oils, which contain 20-25% polyunsaturated fatty acids, produces pro-oxidants such as free radicals and peroxides (Lovell 1989). These compounds react with other nutrients reducing their biological value and availability (Goodard 1996). Ingestion of oxidized fish oils may cause reduced growth, anemia, and nutritional muscular dystrophy in fish (Lovell 1989). Toxic effects of oxidized oils can be reduced by increasing dietary levels of vitamin E. Moreover, to prevent autoxidation, commercial feeds are typically supplemented with synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, or ethoxyquin

(Goodard 1996). When used at a level of 8.75% of the total diet, however, iron concentration of the diet is only 1.5 times that of a high fish meal diet (48.5%).

The objective of this study was to evaluate a SBC product as an ingredient for rainbow trout diets. In this study, 8.75% SBC replaced 27.4% of the herring meal and it reduced the P content of the diet 29.3%. Research issues for fish feed formulation addressed in this project include 1) use of spray-dried blood cells as a partial substitute for fish meal and 2) effect of high iron composition of fish diets containing SBC on fish performance and peroxide content of the feed.

Materials and Methods

Two diets were fed to juvenile rainbow trout for 12 weeks (Table 2). Five replicates were used for each diet, each replicate had 40 fish. Diet 1 (Luzier et al. 1995), which contained

Table 2. Percent composition of reference (diet 1) and experimental diet (diet 2) fed to rainbow trout.

Ingredient	Diet 1	Diet 2
Herring meal, 67.3% protein	47.5	34.5
SBC, 92% protein[a]	0.0	8.75
DL-Methionine[b]	0.2	0.2
Choline Cl 70%	0.2	0.2
Fish oil	7.6	8.35
Soy Flour	8.0	8.0
Feather meal	8.0	8.0
Wheat flour	26.75	30.25
Vitamin mix[c]	1.0	1.0
Mineral mix[d]	0.15	0.15
Sodium chloride	0.5	0.5
Stay-C Dry 25%	0.1	0.1

[a]SBC added at 8.75% of diet replaces 27.4% of herring meal protein.
[b]Methionine, DL, added to meet NRC (1993) requirements (2.6% of protein), based upon analyses of amino acids in ingredients.
[c]Vitamin mixture (Ketola 1983) added at a level of 1% of the diet.
[d]Mineral mixture (Luzier et al. 1995) added at a level of 0.15% of the diet (provided 100 ppm ferrous sulfate to the diet).

47.5% herring meal, was used as the reference diet, because the same formulation had been used in a previous study in this laboratory by Luzier et al. (1995) and it represents a typical high fish meal diet with a modest level of total P (1.19%). Diet 2 contained 8.75% SBC, which replaced 27.4% of the fish meal in diet 1. A commercial laboratory (CN Laboratories, Courtland Minnesota) conducted the analysis of the feed ingredients used in preparation of the diets. Mark Subramanyam (Zeigler Bros. Gardners, PA) formulated the diets based on proximate analysis and amino acid composition of the feedstuffs (Table 3). Pellets were prepared with a laboratory pellet mill without the addition of steam. Diets were pelleted into four sizes: 2.38 mm (3/32 inch) short cut, 2.38 mm (3/32 inch) regular cut, 3.18 mm (1/8 inch) regular cut, and 4.76 mm (3/16 inch) regular cut. All diets were formulated to contain 47% protein (isonitrogenous), isocaloric (based on calculated digestible energy), and to meet other established nutritional requirements for rainbow trout (Table 1).

Experimental fish

Juvenile rainbow trout (initial mean length 81.2 mm), were obtained from Seven Pines Trout Hatchery, Lewis, Wisconsin. When the trout arrived at Iowa State University, Ames, Iowa, they were first placed in five 277-L tanks with water temperature similar to the hauling tank (≈12°C). Water temperatures were gradually raised to 15°C , in each tank over a five-day period, after which the trout were randomly distributed to 30, 100-L rearing tanks at a rate of 45 fish per tank.

The trout were acclimated for 7 days before the start of the feeding trial, at which time the tank populations were reduced to 40 fish per tank. During the acclimation period, all trout were fed the same commercial diet (Salmon Fry, 52% crude protein, Nelson & Sons Inc., Murray, Utah) that the producer had been using.

Water quality and analytical methods

Water inflow to the tanks was regulated to maintain a constant loading (0.4 kg/Lpm) throughout the 12-week feeding trial to ensure that water quality remained above minimum

Table 3. Proximate analysis (%) and amino acid composition of major ingredients used in formulating the experimental diets.

	SBC	Herring meal	Soy flour	Feather meal	Wheat flour
Moisture	8.1	9.8	7.1	8.7	12.1
Protein	93.7	67.3	52.3	86.8	16.1
Fat	0.0	9.54	1.09	4.98	2.72
Fiber	0.5	0.0		0.2	1.2
Ash	4.0	13.1	5.9	1.9	2.0
Iron (mg/kg)	2,569	306	79	501	75
GE (kcal/kg)	5,251	4,804	4,396	5,259	4,007
Amino Acids[a]					
Arginine	4.12	5.63	7.52	6.65	4.65
Histidine	7.32	2.65	2.76	1.53	2.65
Isoleucine	0.63	4.10	4.43	4.67	3.47
Leucine	13.61	7.39	7.72	8.25	6.78
Lysine	8.75	7.62	6.08	2.46	2.25
Methionine + cystine	1.49	3.97	2.89	4.90	3.66
Phenylalanine + tyrosine	8.95	7.29	8.92	8.33	7.72
Threonine	3.12	4.14	4.01	4.49	2.71
Tryptophan	1.54	1.10	1.32	0.67	1.14
Valine	9.12	4.87	4.64	7.41	4.25

[a]Amino acids as % protein.

standards for optimum health of coldwater fish (Piper et al. 1982). To maintain tank hygiene, tanks were cleaned daily by siphoning solids (feed and feces), and biweekly the tank walls were scrubbed to remove biological growth.

Dissolved oxygen was measured daily in each tank with a polarographic probe and meter. Total ammonia nitrogen and pH were measured in each tank twice a week. Total ammonia nitrogen concentration was measured by the nesslerization method (APHA et al. 1992) by using a spectrophotometer. A combination glass electrode and meter was used to measure pH. The percentage of un-ionized ammonia was determined as a function of both pH and temperature from tables given by Thurston et al. (1979).

Feeding

Fish were fed six times a day within a 15-h light period using a mechanical (auger-type) feeder. Daily feeding rates (feed, as fed, as % of the total fish biomass) were calculated in relation to growth rate (cm/day), feed conversion (FC, the ratio of feed to gain), and average total length by the procedure described by Westers (1987). Feeding rates were adjusted once per week. Feeding rates declined from 3.5% in the first week to 3.2% of body weight in the last week.

Apparent digestibility

Apparent digestibility of protein (N = 6.25), phosphorus, and energy of each diet was determined by the chromic oxide indicator method (DeSilva and Anderson 1995). During the last 2 weeks of the study, fish were fed diets containing 0.5% chromic oxide. Fecal samples were collected from the hindmost portion of the rectum (Austreng 1978) from each fish on the last day of the study.

Proximate, amino acid, and chromic oxide analysis and peroxide value of diets; proximate analysis of fish; and protein, gross energy, phosphorus, and chromic oxide content of feces were performed by a commercial laboratory (CN Laboratories, Courtland, Minnesota). Chemical analyses of fish, feed and feces were done according to methods given by the American Oil Chemists Society (AOCS 1993) and Association of Analytical Chemists (AOAC 1975, 1980, 1990) (Table 4).

Table 4. Methods for analysis of trout, diets, and feces samples.

Assay Name	Reference
Moisture	AOCS Ca 2C-25
Protein	AOAC 990.03 15th Edition
Fat	AOAC 7.056 13th Edition
Fiber	AOAC 7.066 13th Edition
Iron	AOAC 968.08 15th Edition
Phosphorus	AOAC 7.103-7.016 12th Edition
Peroxide Value	AOCS Cd 8-53
Pepsin Digestibility	AOAC 7.048 13th Edition

Body composition

Proximate analysis of body composition was done with a random sample of 60 trout at the beginning of the study, and 10 randomly selected trout from each tank (i.e., a composite of 10 fish per replicate) at the end of the study. Body composition comparisons were made on wet basis as suggested by Shearer (1994).

Performance evaluation

Initial total length (mm) and weight (g) was determined from 20 fish randomly selected from the 40 fish in each tank at the beginning of the feeding trial. All trout in each tank were individually weighed and measured at the termination of the 12-week study. At 2-week intervals, 20 fish were randomly selected from each tank to obtain measures of total length and weight. The biweekly samples were used to calculate growth rates. Also, at the beginning and every two weeks, all fish were weighed together to obtain the tank biomass to determine the amount of feed to offer based on percent of body weight per day (BW%/d).

Whenever fish were sampled, inflow water was reduced by 50%, and before the fish were captured, they were sedated by the addition of 20 mg/l of tricane methane sulfonate (Finquel™, Argent Chemical Laboratories, Redmond, WA) to the tank. After capture, fish were placed into a buffered (25 mg/L sodium bicarbonate, $NaHCO_3$) solution of 1% NaCl, and 60 mg/L of Finquel™ to obtain complete anesthesia before individual measurements were obtained. The NaCl was added to reduce chloride loss that is typical of fish following handling, and the bicarbonate to prevent a pH drop from the acidic nature of the anesthetic.

Growth rates were calculated as g/day, specific growth rate (SGR) (Hopkins 1992), mm/day, and unit growth rate (UGR; cm/day/°C) (Westers 1987). SGR was calculated as $\{[Ln(w_f)-Ln(w_i)]/t\} \times 100$, where w_f = final weight, w_i= initial weight, and t = length of culture period. The condition factor (K), an expression of body condition, was calculated as weight $\times 10^5$/length.

Net protein utilization (NPU) was calculated as protein gain divided by total protein fed, where protein gain is the final protein content minus the initial protein content. NPU was chosen as the measure of protein utilization rather than protein efficiency ratio (PER) because NPU measures the amount of protein deposition, whereas PER measures weight gain that can also be attributed to fat accumulation (DeSilva and Anderson 1995). Phosphorus retention was calculated as [(fish weight gain) × (fish P concentration)] / [(weight feed fed) × (feed P concentration)] × 100 (Heinin et al. 1993). Iron retention was calculated by substituting iron concentration for P concentration in the P retention equation.

Statistical analysis

Measures of performance, including weight gain (g/day), length gain (mm/day), UGR, SGR, G, NPU, FC, and K, were compared with a two-tailed, unpaired t-test. The 0.05 level was used to indicate statistical significance of the t-tests. Experimental variability was reported as coefficient of variability (% CV = standard deviation ÷ mean × 100).

Results

Diet formulation

As formulated, the diets were nearly isonitrogenous, 48.5 and 48.0% crude protein, and isocaloric, 4,886 and 4,912 kcal/kg gross energy, for diets 1 and 2, respectively. The diets with SBC contained 27.4% less herring meal and 13.1% more wheat flour than the reference diet. Amino acid composition of both diets was similar (Table 5), but the test diet with 8.75% SBC had slightly lower content of isoleucine but higher concentrations of histidine, leucine, and valine than the reference diet (diet 1) without SBC. All diets equaled or exceeded the established amino acid requirements for rainbow trout (NRC 1993).

The phosphorus content of the SBC diet (diet 2, 0.92% P) was 22.6% lower than the P content of the reference diet (diet 1, 1.19% P). Diet 2 had 48.8% more iron content (433 mg/kg) than diet 1 without SBC (291 mg Fe/kg).

Table 5. Proximate analysis (wet weight basis) and amino acid composition of the test diets. Diet 1 consisted of 47.5% herring meal and diet 2 consisted of 34.5% herring meal and 8.75% SBC.

Component	1	2	NRC[c]	Difference diet 2-NRC
Moisture	9.3	9.7		
Protein	48.5	48.0	38	10
Fat	14.3	13.5		
Fiber	1.0	1.5		
Ash	7.9	6.3		
NFE[a]	19.0	21.0		
Phosphorus	1.19	0.92	0.6	0.32
Iron (mg/kg)	291	433	60	373
Gross energy (kcal/kg)	4,886	4,912		
Digestible energy (kcal/kg)[b]	3,977	3,927	3,600	327
Amino acids[d]:				
Arginine	5.91	5.58	4.0	1.58
Histidine	2.28	3.20	1.8	1.40
Isoleucine	4.17	3.72	2.4	1.32
Leucine	7.51	8.80	3.7	5.10
Lysine	6.55	6.52	4.7	1.82
Methionine	2.77	2.26		
Methionine + cystine	4.12	3.84	2.6	1.24
Phenylalanine	4.18	4.62		
Phenylalanine + tyrosine	6.95	7.87	4.7	3.17
Threonine	3.97	3.86	2.1	1.80
Tryptophan	0.51	0.53	0.5	0.03
Valine	4.98	6.21	3.2	3.01

[a]Nitrogen free extract (NFE) =100-(moisture + protein + fat + fiber + ash).

[b]Estimated form ingredient composition by M. Subramanyam (Zeigler Brothers, Inc., personal communication).

[c]NRC (1993).

[d]Amino acids as % of protein.

Growth and condition

None of the measures of growth in fish length differed significantly ($P > 0.05$) between treatments (Table 6). Differences in initial and final condition factor between the diets were small, but for both diets, final body condition was substantially greater than the initial.

None of the measures of weight gain differed significantly between treatments (Table 7). Weight gains ranged from 0.65 to 0.69 g/day and exceeded 800% of the initial weight in both

Table 6. Comparison of growth measures for length of rainbow trout fed the reference (diet 1) or experimental diet (diet 2). Diet 1 consisted of 47.5% herring meal and diet 2 consisted of 34.5% herring meal and 8.75% SBC.

Diet	Initial mean length (mm)	Growth (mm/day)	Growth (UGR)[b]	Condition, K[c]	
				Initial	Final
1	80.9 ± 0.36[a] (1.0)	1.00 ± 0.02 (4.2)	0.0057 ± 0.0001 (4.2)	1.18 ± 0.02 (3.5)	1.44 ± 0.01 (1.9)
2	81.9 ± 0.77 (2.1)	0.96 ± 0.01 (2.7)	0.0055 ± 0.0001 (2.7)	1.17 ± 0.02 (4.4)	1.42 ± 0.01 (1.9)
P-value	0.266	0.093	0.093	0.893	0.303

[a]Mean ± standard error, and coefficient of variability in (CV).
[b]Unit growth rate (UGR) = daily length increase (ΔL, cm) per unit of temperature (Westers 1987).
[c]Condition (K) = weight (g) × 100,000 ÷ total length $(mm)^3$.

Table 7. Comparison of growth measures for weight of rainbow trout fed the reference diet (diet 10 and experimental diet (diet 2). Diet 1 had 47.5% herring meal and diet 2 had 34.5% herring meal and 8.75% SBC.

Diet	Initial mean weight	g/day	SGR[b]	% Weight gain	FC
1	6.2 ± 0.13[a] (4.7)	0.69 ± 0.02 (5.0)	2.78 ± 0.04 (3.1)	932.7 ± 33.6 (0.81)	0.98 ± 0.03 (8.1)
2	6.5 ± 0.27 (9.5)	0.65 ± 0.01 (4.0)	2.67 ± 0.04 (3.7)	842.0 ± 34.4 (0.91)	1.02 ± 0.01 (1.5)
P-value	0.47	0.41	0.096	0.096	0.370

[a]Mean ± standard error, and coefficient of variation in (CV).
[b]Specific growth rate (SGR).

treatments. FC values were near 1.0 for both diet treatments and differences between diets were small.

Nutrient utilization

Differences in net protein utilization (NPU) between fish in diet treatments 1 and 2 were not statistically different (Table 8). Phosphorus retention was significantly higher for fish fed the

Table 8. A comparison (t-test) of protein, phosphorus, and iron utilization by trout fed diet 1, which consisted of 47.5% herring meal, and diet 2, which consisted of 34.5% herring meal and 8.75% SBC.

Diet	NPU[a]	Phosphorus retention	Iron retention
1	33.0±1.24[b] (7.6)	34.1 ± 0.99 (0.06)	5.8 ± 0.60 (0.21)
2	31.8±0.53 (3.7)	42.0 ± 1.72 (0.09)	4.2 ± 0.28 (0.15)
P-value	0.375	0.007	0.032

[a]Net protein utilization (NPU).
[b]Mean ± standard error, and coefficient of variation in (CV).

SBC diet (Table 8). Iron retention was low for both groups of fish but it was significantly lower in fish fed the SBC diet than the reference diet.

Body composition

Differences in body composition (moisture, fat, ash, iron, or phosphorus) of fish fed diets 1 and 2 at the end of the experiment were not statistically significant (Table 9). The final protein and fat composition of fish fed both diets was greater than the initial composite sample. Moisture and iron concentration of trout fed both diets decreased from initial values over the 12-week feeding trial.

Digestibility of diets

Differences between diet treatments in mean apparent digestibility coefficients (ADC) for protein are small, but because variability was low the differences in the means were statistically significantly (Table 10). Diet 2 containing SBC had a significantly higher ADC for protein (89.7% digestible protein) than diet 1 without SBC (88.1%) ($P = 0.02$). Energy and phosphorus ADCs were not significantly different between diets (Table 10). Digestible protein and energy were nearly identical in the diets. Pepsin digestibility of protein of both diets was high, and they were similar.

Table 9. Comparison of body composition of rainbow trout at the end of the 12-week feeding trial fed the reference diet and experimental diet. Initial body composition is given for reference. Diet 1 consisted of 47.5% herring meal and diet 2 consisted of 34.5% herring meal and 8.75% SBC.

Diet	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Phosphorus (%)	Iron (mg/kg)
Initial[a]	77.8	13.8	5.04	2.2	0.35	23.3
1	71.1±0.47[b] (1.3)	15.6±0.17 (2.2)	11.0±0.52 (9.5)	2.3±0.07 (6.2)	0.40±0.01 (7.4)	16.5±1.26 (15.3)
2	71.7±0.11 (0.4)	15.4±0.23 (3.3)	10.8±0.42 (8.7)	2.1±0.74 (7.7)	0.39±0.01 (7.7)	18.4±1.12 (13.6)
P-value	0.189	0.586	0.809	0.127	0.775	0.299

[a]Mean ± standard error, and coefficient of variability in (CV).
[b]Initially a 60-fish composite sample was analyzed, the final body composition of each diet treatment is a mean of 5 replicate samples of 10 fish each per diet.

Table 10. Pepsin digestibility and apparent digestibility coefficients (ADC) of the protein, phosphorus, and energy in diet 1, which consisted of 47.5% herring meal and diet 2, which consisted of 34.5% herring meal and 8.75% SBC. There were five replicate measurements of each coefficient.

	Diet		P-value
	1	2	
Pepsin Digestibility	96.6	95.7	
ADC			
Protein	88.1±0.38[a] (0.9)	89.7±0.40 (1.0)	0.023
Phosphorus	53.1±2.0 (8.46)	51.6±2.18 (9.4)	0.624
Gross Energy	84.2±0.82 (2.17)	82.4±0.97 (2.64)	0.202
DP (%)	42.7	43.0	
DE (kcal/kg)	4,114.0	4,047.5	

[a]Mean ± standard error, and coefficient of variability in (CV).

Peroxide values

Peroxide value (PV, expressed as meq/kg of extracted fat) of the diets at the start of the study were greater in both diets than at the end of 13 weeks (Table 11). Peroxide content of diet 2 declined from 1.8 meq/kg initially to 0.82 meq/kg at the end of the study. Peroxide value of diet 1 was lower at the beginning (1.1 meq/kg) and lower at the end (0.52 meq/kg) of the study than the test diet containing SBC (diet 2).

Water quality

Differences between treatments in dissolved oxygen, total ammonia nitrogen (TAN), and un-ionized ammonia of water samples from culture tanks were not significant (Table 12). Overall mean temperature was 17.4°C (range 15.9 to 18.6°C).

Survival

Mortality throughout the trial was low, only one of 200 fish in each treatment died in the 12 week study.

Table 11. Peroxide value (meq/kg of extracted fat) of feed at the beginning and end of the study.

	Feed	
Diet	Begin	End
1	1.1[a]	0.52
2	1.8	0.82

[a]Peroxide values between diets could not be compared statistically because there were no possibilities for replicates of the diets.

Table 12. Comparison of water quality in culture tanks during the feeding trial.

Diet	Temperature	DO (mg/L)[a]	TAN (mg/L)	UA (mg/L)[b]
1	17.4 ± 0.2 (0.003)	8.1 ± 0.05 (0.013)	0.25 ± 0.011 (0.09)	0.0038 ± 0.00017 (0.09)
2	17.4 ± 0.2 (0.003)	8.0 ± 0.06 (0.016)	0.27 ± 0.006 (0.05)	0.0039 ± 0.00013 (0.07)
P-value		0.309	0.150	0.826

[a]Mean ± standard error, and coefficient of variability in (CV). Temperature and dissolved oxygen (DO) is the treatment mean based on 5 replicate tanks, each the mean of 83 samples. TAN and UA are the treatment means based on 5 replicate tanks sampled 20 times during the study.

[b]UA, unionized ammonia.

Discussion

Diet composition and digestibility

The 8.75% SBC in experimental diet (diet 2) replaced 27.4% of the fish meal present in the reference diet (diet 1). Both diets had an amino acid profile that met the NRC (1993) requirements for rainbow trout. Diet 2 had higher values for protein, total P, iron and all amino acids than NRC (1993) requirements. The major amino acid difference between diet 2 and NRC (1993) requirements was the much higher leucine and phenylalanine + tyrosine content of diet 2. Regarding the amino acid composition of the SBC, the high lysine content (8.75% of protein) of SBC would be useful to balance diets high in plant meals, which are typically low in lysine, –lysine is 2.94% of protein in corn meal, 3.16 in soybean meal, and 2.25% in wheat flour.

Diet 2 had a slightly greater apparent protein digestibility (89.7%) than the reference diet (88.1%). The digestible protein (DP) content of the diets is therefore 42.7% for diet 1 and 43.0% for diet 2. Digestible energy (DE) of diet 1 was 4,110.0 kcal/kg, only 1.6% greater than diet 2, which contained 4,047.5 kcal/kg. DE and DP of both diets were greater than the NRC (1993) recommendation of 3,600 kcal DE/kg and 34% DP for rainbow trout. A DP:DE ratio of 9.4 is calculated from the NRC (1993) requirements, which is lower than the DP:DE ratio of diet 1 (10.4) and diet 2 (10.6).

The apparent digestibility of Norse-LT94 herring meal is 85.8% (Johnsen and Wandsvik 1991), and crude protein digestibility of herring meal is 92% (Cho 1990). The crude protein digestibility coefficient of spray-dried blood is 99% (Cho 1990). LT fish meals are considered to be of better quality than traditionally processed fish meals (Jobling 1993). Luzier et al. (1995) determined that apparent protein digestibility of diets containing 5.6% or 11.1% spray-dried blood were 83.0% and 79.5%, respectively. The reference diet in that study had 78.8% apparent digestible protein (Luzier et al. 1995). Both diets in the current study had protein digestibilities greater than those measured by Luzier et al. (1995). Asgard and Austreng (1986) also found that acid-ensiled blood in moist salmonid diets had a higher protein digestibility than fish meal.

Pepsin digestible protein (PDP) value of the reference diet was 96.6% compared with 95.7% for the SBC diet. Both values are higher than PDP values reported by Heinen et al. (1993) for four commercial diets that had PDP values of 79.9% to 88.7%. Higher PDP values of the diets in this study may be due to use of higher quality ingredients than used in commercial diets, and there are potential effects on digestibility related to manufacturing process.

Phosphorus digestibility in the reference diet (53.1%) and test diet (51.6%), were greater than phosphorus digestibility values reported by Luzier et al. (1995), which ranged from 30.3% for a diet containing 5.6% spray-dried blood (1.22% dietary phosphorus) to 45.2% for a diet containing 22.7% spray-dried blood (0.84% dietary phosphorus). In that study, P digestibility increased with decreasing concentration of spray-dried blood. Phosphorus retention is correlated with differences in phosphorus digestibility, and the diet with SBC not only had a lower P content, but substantially greater P retention. Phosphorus retention for fish fed the SBC diet was 42.0% compared with 34.1% for fish fed the reference diet, a difference of 23.3%. Heinen et al. (1993) reported 21.4% to 36.3% phosphorus retention by rainbow

trout fed commercial trout feeds and Ketola (1991) calculated phosphorus retentions of 16 to 18% when diets contained 1.3% to 1.6% P.

Dietary iron levels of diet 2 prepared with SBC greatly exceeded the minimum requirement of 60 ppm Fe suggested by the NRC (1993), but iron content of the fish declined over the 12-week experiment. Our measure of iron retention suggested that iron retention is metabolically controlled and excess iron was eliminated. The iron contained in feedstuffs used in formulating these diets would have provided enough iron without the standard supplement of 100 ppm ferrous sulfate added to ensure adequate digestible iron for the reference diet. Iron content of fish and feed is discussed further in the section on body composition.

Growth and performance

Unit growth rates (UGR) for diets 1 (0.0057) and 2 (0.0055) did not differ, and they were similar to UGRs ranging from 0.0054 to 0.0058 reported by Luzier et al. (1995), but they were greater than the 0.00529 reported for rainbow trout at 16°C (data of Dwyer et al. 1981 as modified by Westers 1987). Westers (1987) stated that the maximum UGR for rainbow trout with a condition factor and a feed conversion of 1.0 was 0.00667, which is higher than the UGRs in the current study and Luzier et al. (1995). UGRs that we calculated from data reported by Heinen et al. (1993) ranged from 0.0066 to 0.0079 for Kamloops strain rainbow trout fed commercial diets. Feed conversion ratios of trout fed commercial diets in the study by Heinen et al. (1993) ranged from 1.24 to 1.55 compared with 0.98 to 1.02 in the present study.

Luzier et al. (1995) found that a diet containing 22.7% spray-dried blood (APC 300) could replace 65.3% of herring meal protein in rainbow trout diets with performance equal to that of the reference diet containing 47.5% herring meal. The results of the current study indicate that removal of the plasma from spray-dried red blood cells (SBC) did not affect fish performance when SBC were added at a level of 8.75% of the diet. All measures of growth, condition, feed conversion, and protein efficiency (NPU) of rainbow trout fed the SBC diet were not

significantly different from the reference diet. Thus, as little as 8.75% SBC replaced up to 27.4% of the fish meal protein in rainbow trout diets without compromising performance. An additional benefit is that the SBC diet (0.92% P) contained 22.6% less phosphorus than the reference diet (1.19% P) and the fish fed this diet retained a substantially greater percentage of P than the reference diet. Trout fed the reference diet digested 53.1% of the P compared with 51.6% in the reference diet. Both diets exceeded the minimum P requirement for rainbow trout of 0.54% available phosphorus (Ketola and Richmond 1994). The SBC diet also contained less phosphorus than reported for some commercial trout diets: 1.35% for Oregon Moist Pellet (Ketola and Harland 1993); 1.19% for Balls Trout Grower; 1.15% for Zeigler High Performance Trout Grower (Heinen et al. 1993). Of course, diet formulations for proprietary diets undergo rapid changes, and these measures of phosphorus content may not represent current phosphorus content for diets by these companies.

Body composition

Diet did not influence the final body composition of trout in this study, however, as fish size increased from beginning to end of the study, some changes in body composition were noted. Whole body moisture and whole body lipid content are inversely related (Shearer 1984). At the end of the 12 weeks, whole body moisture decreased from 77.8% to about 71% and fat increased from 5.0% in the initial sample to about 11%. Protein also increased from 13.8% to about 15.5% during the study. Shearer (1994) stated life cycle stage, size, and energy intake as the primary factors that influence fish body composition.

Iron content of fish decreased during the 12-week study from 23.3 mg Fe/kg initially to 16.5 mg Fe/kg in fish fed the reference and 18.4 in fish fed the SBC diet. On the other hand, whole body phosphorus content increased from 0.35% initially to 0.40% and 0.39% in fish fed the reference diet (diet 1) and the SBC diet (diet 2), respectively. Bjornevik and Maage (1993) reported a decrease in body iron content during growth of Atlantic salmon Salmo salar from 16.3 mg Fe/kg in 31g fish to 9.5 to 12.5 mg Fe/kg in 70 g fish.

The difference in final iron content of rainbow trout fed the two diets was not significant, even though the diet with SBC contained 433 mg Fe/kg iron compared with 291 mg Fe/kg iron in the reference diet, a difference of 49%. The findings of the current study support those of Andersen et al. (1996) who suggested that there is a regulatory mechanism for iron balance, which is maintained largely by regulation of iron absorption according to the body needs. However, experiments by Desjardins et al. (1987) and Bjornevik and Maage (1993) with rainbow trout and Atlantic salmon do not support a hypothesis for regulation of iron absorption. Desjardins et el. (1987) found that rainbow trout fed diets with modest levels of iron (25-86 mg Fe/kg diet) had iron content that ranged from 24 to 30 mg Fe/kg dry weight, but trout fed high iron diets, ranging from 265 to 5,934 mg Fe/kg, had body iron content of 265 to 153 mg/kg dry weight. Bjornevik and Maage (1993) reported that Atlantic salmon fed a diet containing 33 or 53 mg Fe/kg had lower body iron content than salmon receiving a 160 mg/kg iron diet.

Shearer (1984) found that whole body mineral concentrations of rainbow trout averaged 12 $\pm$ 3.8 mg Fe/kg for iron and 0.48% $\pm$ 0.09 phosphorus in fish weighing 10-1822 g (Table 13). Shearer (1984) presented equations to predict the wet weight or "body burden" of several elements (calcium, copper, iron, magnesium, manganese, sodium, phosphorus, strontium, and zinc) in rainbow trout. Body iron and phosphorus concentrations increase at the onset of feeding throughout the juvenile growth period (0.14 g to 17g), then decrease during the post-juvenile period (17g to 1382g) (Shearer 1984). Diets used by Shearer (1984) contained an average 367 mg/kg iron, which is intermediate to the diets used in the current study, and 2.06% phosphorus, which is about twice the average phosphorus content of both diets used in the current study.

The initial iron content of rainbow trout in the present study was 23.33 mg Fe/kg, which is higher than the calculated value of 16.5 mg Fe/kg for the average initial weight of fish fed both diets using the Shearer (1984) equation for trout 0.14 to 17g, $\log_{10}$ body burden (μg) = 1.08 +

1.17 × ($\log_{10}$ fish wet weight(g)). In our study, final iron content was 16.5 and 18.4 for fish fed diets 1 and 2, respectively. These values were close to the calculated values of 16.0 for fish fed diet 1 and 15.8 for fish fed diet 2 for similar sized trout using the equation from Shearer (1984) for trout greater than 17g: $\log_{10}$ body burden (μg) = 1.46 + 0.856 × $\log_{10}$ fish wet weight(g).

Table 13. Body composition of rainbow trout.

Moisture	Protein	Fat	Ash	Phosphorus	Fish size (g)
68[a]	15.1	13.9	2.0		59.3
72.2[b]	14.2	10.9	2.1	0.37	
75.2[c]	16.4	5.2	3.1		38.2
67.1[c]	17.6	12.9	2.5		135.4
				0.51[d]	28.0
				0.49[d]	28.0
				0.48[e]	10-1882
70.2[f]	16.3	9.2		0.44	137
69.2[f]	17.4	10.9		0.39	185

[a]Luzier et al. (1995). Value presented are averages of two diets containing 5.6 and 11.1% spray-dried blood.
[b]Asgard et al. (1991). Size of fish at sampling not given.
[c]Oliva-Teles et al. (1994). First row is a pretrial fish sample. Second row of values are for fish fed a reference diet (57% fish meal).
[d]Ketola and Richmond (1994). Fish were fed a semi-purified diet containing 0.91 and 1.21% non-phytin P.
[e]Shearer (1984)
[f]Heinen et al. (1993), fish were fed commercial diets containing 1.19% and 1.49% total P.

The organs of juvenile trout exhibit positive allometric growth, that is, some organs or tissues grow at a faster rate than the whole body (Weatherly and Gill 1983). The liver contains 89 mg/kg iron, which is much higher than the whole fish content of 12 mg/kg (Shearer 1984). Weatherly and Gill (1983) found that the weight of the liver of fingerling trout increased relatively more rapidly than the carcass, exhibiting positive allometry, but after the fingerling stage, the liver grows at a slower rate than the whole body, causing a decline in the whole body iron concentration of the post-juvenile trout. The findings of Weatherly and Gill (1983) help explain our observation that iron content declined from beginning to end of the study.

Iron is an essential micronutrient, it is present in heme compounds, heme enzymes, and nonheme compounds (NRC 1993). Feed is the major source of iron, although fish can absorb some soluble iron (Fe^{2+}) from the water through their gills; however, the concentration of dissolved (soluble) iron in water is low (Lovell 1989). NRC (1993) dietary recommendations for iron requirements for trout are 60 mg/kg of diet; however, this is lower than typical commercial diets. The Canadian practice is to add an iron supplement (ferrous sulfate) to commercial trout diets (Hilton and Slinger 1981). Desjardins et al. (1987) reported that commercial trout diets contain between 200 and 1,000 mg iron/kg feed because of the presence of fish meal and blood meal. The iron content of Norwegian commercial fish-meal diets is in the range of 51 to 515 mg/kg (Andersen et al. 1996). A commercial diet used in a study of the hypoferremic response in rainbow trout (Conglton and Wagner 1991) had an iron content of 208 mg Fe/kg of diet.

Sealey et al. (1997) studied the relationship between the level and source of iron, and the immune response of channel catfish against Edwardsiella ictaluri. Channel catfish fed iron deficient diets were more susceptible to disease than fish fed diets containing 30 mg Fe/kg, however, iron supplementation of 180 mg ferrous sulfate/kg (possible iron overload for channel catfish) may have increased susceptibility to E. ictaluri.

In rainbow trout toxicity signs (reduced growth, increased mortality, and histopathological changes in the liver) develop when dietary iron exceeds 1,380 mg Fe/kg in diets with a low level of diet rancidity (Desjardins et al. 1987). In the present study, the diet with 8.75% SBC, contained 433 mg Fe/kg diet, 48.8% more iron than the reference diet (291 mg Fe/kg), but there were no signs of iron toxicity in the diets with SBC, and none would be expected unless the diets contained high levels of rancidity from lipid peroxidation (Desjardins et al. 1987).

Whole body phosphorus content in fish fed both diets was lower (0.49%) than the value calculated by the equation given by Shearer (1984). In the current study, phosphorus content was initially 0.35% and it increased to about 0.40% at the end of the trials, lower than 0.49%

calculated from the equation by Shearer (1984) for trout of similar weight ($\log_{10} P = 3.75 \times 0.965 \log_{10}$ wet weight (g)). The diets fed in the Shearer (1984) study had about twice the phosphorus content as the diets fed in the study reported here, which may affect P-content of the body.

Peroxide values

Peroxide values (PV, meq/kg of extracted fat) at the beginning and end of the study were slightly higher in the diet containing SBC than the diet without SBC. Peroxide values decreased by about half during the 12 weeks. The peroxide values are all very low and within normal analytical variation (Joel Sieh, CN Laboratories, personal communication). The decrease in peroxide values may have resulted from interference in the fat extraction process (Reimann 1994). Or the decrease could be explained by the transition of peroxides into secondary oxidation products which are not determined by the peroxide value test but are determined by the thiobarbituric acid value, anisidine value, and the Kreis test (Burkow et al. 1992). Reimann (1994) stated that there is a general agreement that the fat extraction process is likely to modify the fat and peroxide value of the fat; therefore, the same fat extracted from a feed may show significantly different peroxide values.

Antioxidants, ethoxyquin or butylated hydroxytoluene (BHT), were present in the vitamin premix, herring meal, and fish oil used in the formulation of the diets (Tim Markey, Zeigler Brothers Inc., personal communication), the calculated antioxidant level in the diets was about 0.02%. Generally, antioxidants are added to formulated diets at 0.015% (Jobling 1993).

Cho (1990) stated that a fish oil should have peroxide values less than 5 meq/kg. The peroxide values for fat extracted from both diets in the current study were lower than 5 meq/kg indicating that the feeds met the peroxide value quality standard. In an experiment by Desjardins et al. (1987) a fish oil with a peroxide value of 4.3 mEq/kg was considered "fresh" and a peroxide value of 56.9 mEq/kg was considered "poor" quality.

Typically, fish meal is less expensive than spray-dried blood. However, in aquaculture situations where phosphorus effluent is a concern, the increased cost of incorporating spray-dried blood cells in the feed formulation at the expense of fish meal may be justified by the concomitant reduction in dietary phosphorus content. Current price of herring meal (72% crude protein) is about $760/ton (Tim Markey, Zeigler Brothers Inc., personal communication), the October, 1997 average price of menhaden fish meal (64.5% crude protein) was $560/ton (FOB Kansas City) (Feedstuffs 1997a, 1997b, 1997c, 1997d). The estimated cost of spray-dried blood cells (92.0% crude protein) is $800/ton. However, the cost of protein in menhaden fish meal and spray-dried blood cells is the same at $0.956/kg of protein, herring meal protein costs $1.16/kg. Using the current prices of herring meal, SBC, fish oil, and wheat flour, the diet with 8.75% SBC would cost $0.017/kg less than the standard diet (Table 14).

Table 14. Estimated savings (per 100 kg of diet) by replacing 13% herring meal with 8.75% spray-dried blood cells (SBC), fish oil, and wheat flour in a rainbow trout reference diet.

Ingredient	% change	$/kg	Savings
Herring meal	-13.0%	0.836[a]	-10.87
SBC	+8.75%	0.880[b]	+7.70
Fish oil	+0.75%	0.660[a]	+0.50
Wheat flour	+3.5%	0.268[a]	+0.94
Total			$-1.73/ 100 kg

[a](Tim Markey, Zeigler Brothers Inc., personal communication).
[b](Fang Chi, American Protein Corporation, personal communication).

The spray-dried blood cell product (SBC) evaluated in this study was a suitable ingredient for formulating diets for rainbow trout. None of the differences in growth comparisons between the diet with SBC and the reference diet were statistically significant. The phosphorus content was reduced by 29% in the diet containing SBC. This was accomplished by adding 8.75% SBC at the expense of 13% of the herring meal, a replacement of 27% of the herring

meal protein. Addition of SBC slightly increased protein digestibility of the test diet. The increased iron content of the SBC diet did not affect trout performance and the peroxide value of the SBC diet remained low. At current prices of herring meal and spray-dried blood cells, replacing 27.4% of the herring meal protein with SBC would slightly reduce the cost of the diet.

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GENERAL CONCLUSION

There is a need to reduce the content of or find replacements for fish meal in fish diets for economic and resource depletion reasons, and to obtain the objective of using diets with phosphorus content not in excess of the dietary requirement of the fish. For these reasons, alternative protein sources are being sought for fish meal. Spray-dried blood is a high protein, high lysine, and highly digestible feedstuff which is also low in phosphorus. These nutritional qualities make it a desirable candidate to reduce fish meal content of the fish feeds. However, spray-dried blood has a high iron content, which can greatly increase the iron levels in the final diet. Iron content of fish feeds is a concern because a high iron content in feed may 1) increase lipid autoxidation and 2) suppress the immune system and increase disease susceptibility of fish.

In this study, rainbow trout Oncorhynchus mykiss were fed a diet containing 34.5% herring meal and 8.75% spray-dried blood cells (SBC) as a partial replacement for herring meal. Trout fed the SBC diet grew as well as trout fed a reference diet containing 47.5% herring meal and no spray-dried blood cells. Trout fed the reference diet gained 1.0 mm/day and 0.69 g/day compared with 0.96 mm/day and 0.65 g/day for the fish fed the test diet. Feed to gain ratios were 0.98 and 1.02 for the reference diet and test diet, respectively. Mortality was 0.5% (1 fish/200) in each treatment. Apparent protein digestibility was 89.7% for the diet containing SBC compared with 88.1% for the reference diet, a small but statistically significant ($P = 0.02$) difference. Iron content of the test diet containing SBC was 433 mg Fe/kg. Whole body content of iron decreased in fish fed both the reference and the test diets in the 12-week experiment from 23.3 mg Fe/kg initially, to 16.5 mg/kg in the reference group and to 18.4 mg/kg in the test group at the end of 12 weeks. There was no indication that lipid peroxidation reduced the performance of trout fed either diet.

This experiment shows that SBCs are suitable for partial replacement of fish meal in rainbow trout diets. The protein in the diet containing SBC was slightly more digestible than

the protein in the reference diet. The 8.75% substitution level of SBC replaced 27.4% of the fish meal and reduced the phosphorus content of the diet by 29.3%. There was no observable palatability problem in feeding the blood concentrate. The higher levels of iron in the diet containing SBC did not have any adverse affect on trout performance.

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